Summary of Ph.D. thesis

Synthesis and structural investigation of 17β-Pyrazolyl and -pyrazolinyl steroids

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1. Introduction and aims

The synthesis of heterocycle bearing steroids is widely studied in the last two decades because of their favorable biological activities. It has been proved for several 17-*exo*-heterocyclic steroids that these compounds exert inhibition on key enzymes regulating the androgen biosynthesis. This ability makes them possible candidates for the treatment of *benign prostatic hyperplasia* and *prostatic carcinoma.* The latest researches reported the antiproliferative activity of heterocyclic steroids on several human cancer cell lines.

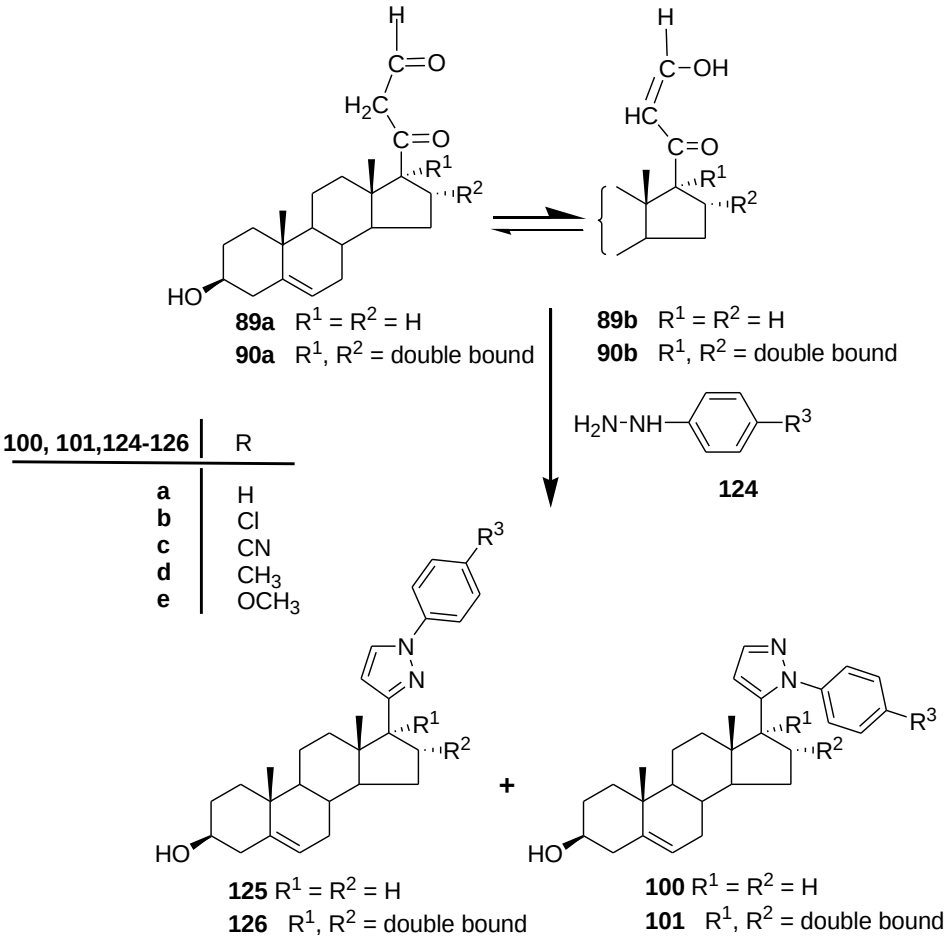
The aim of our research was the synthesis of novel androst-5-ene and androsta-5,16-diene derivatives that contain pyrazole and pyrazoline rings in position 17 of the steroid skeleton. It was also our aim to study the regio- and stereoselectivity of the reactions and to separate the isomers formed during the syntheses with chromatography. We planned NMR spectroscopic investigations for structure assignations of the synthesized compounds.

2. Experimental methods

Most of the reactions were carried out in millimolar scale, and were monitored by thin-layer chromatography. The crude products were purified by flash chromatography, their structures were investigated by ^{1}H and ^{13}C NMR techniques. In the case of some compounds, 2D NMR experiments (COSY, HSQC, NOESY) were used for the structure determination.

3. Scientific results*

3.1 We found that the ring closure reaction of 3β-hydroxy-21-hydroxymethylidenepregn-5-en-20-one (**89**) with phenylhydrazine (**124a**) lead to a mixture of regioisomers despite of the earlier observations in the literature (Scheme 1). In parallel to the 17β-(1-phenyl-5-pyrazolyl)androst-5-en-3β-ol (**100a**), 17β-(1-phenyl-3-pyrazolyl)androst-5-en-3β-ol (**125a**) also formed in considerable amount.



Scheme 1

3.2. The reaction was carried out with the hydrochloride salts of *p*-substituted phenylhidrazines (**124b**–**e**), and the formation of the regioisomers (**125b**–**e**, **100b**–**e**) was still observed. All of the 5-pyrazolyl androstenes (**100a**–**e**) proved to be more

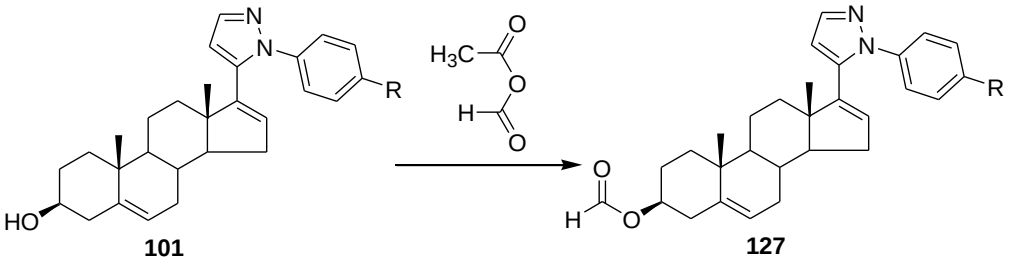
* The numbering of the compounds accords with that in the Ph.D. Thesis

polar than the corresponding 3-pyrazolyl derivatives (**125b**–**e**). The isomeric pairs were separable by column chromatographyc method in all cases.

3.3. It was found that the isomeric ratio strongly depends on the substituent applied in the *p*-position. Substituents with electron-donating character (e.g. *p*-OCH_3) increased the amount of the 3-pyrazolyl regioisomers, while in the case of the electron-withdrawing substituents (*p*-Cl, *p*-CN), the 5-pyrazolyl derivatives were the main isolable products.

3.4. The dependence of the isomeric ratios on the reaction conditions was also observed. Ring closing reactions carried out with synton equivalent **89** in dichloromethane, catalyzed by *Lewis* acid resulted in increased amounts of the 3-pyrazolylandrostenes (**125a**–**e**).

3.5 The ring closing reactions with phenylhydrazine and its *p*-substituted derivatives were performed with 3β-hydroxy-21-hydroxymethylidenepregna-5,16-dien-20-one (**90**), which was obtained from a *Claisen* condensation carried out at low temperature in pyridine. We established that the regioisomers - previously observed for the corresponding saturated compounds - were also formed in these conversions (**101a**–**e**, **126a**–**e**). However the ratio of the 5-pyrazolyl isomers was found to be significantly higher (Scheme 1). Furthermore we detected the formation of 17-(1-phenyl-5-pyrazolyl)androsta-5,16-dien-3β-yl formate, and its substituted derivatives (**127a**–**e**). This could be explained by esterification with an anhydride resulting from the *retro-Claisen* condensation of the starting material (Scheme 2).



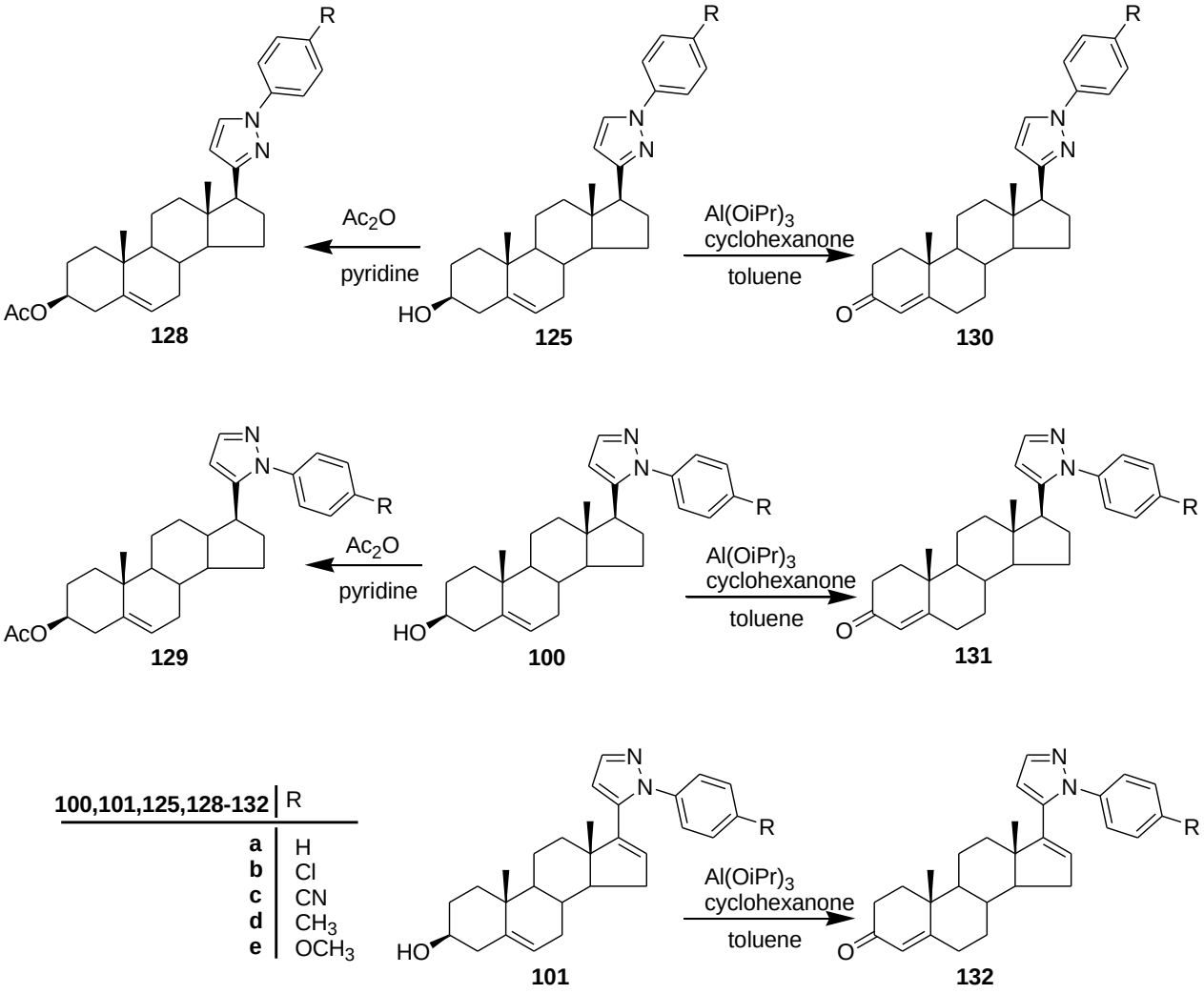
101 **127**

101, 127	R
a	H
b	Cl
c	CN
d	CH_3
e	OCH_3

Scheme 2

3.6 The pyrazolylandrost-5-ene and -androsta-5,16-diene derivatives exhibited characteristic differences in their optical rotations. We have observed that the $[\alpha]_D^{20}$ values of compounds **100a**–**e** and **101a**–**e** were always more negative than those of their corresponding **125a**–**e** and **126a**–**e** isomers.

3.7 The 3β-acetoxy derivatives (**128a**–**e**, **129a**–**e**) of the synthesized steroids bearing hydroxy group in position 3 (**100a**–**e** and **125a**–**e**) were also prepared and characterized. *Oppenauer* oxidation of compounds **125a**–**e**, **100a**–**e** and **101a**–**e** lead to the corresponding Δ^4-3-ketosteroids (**130a**–**e**, **131a**–**e**, **132a**–**e**) without the degradation of the heterocycle (Scheme 3).

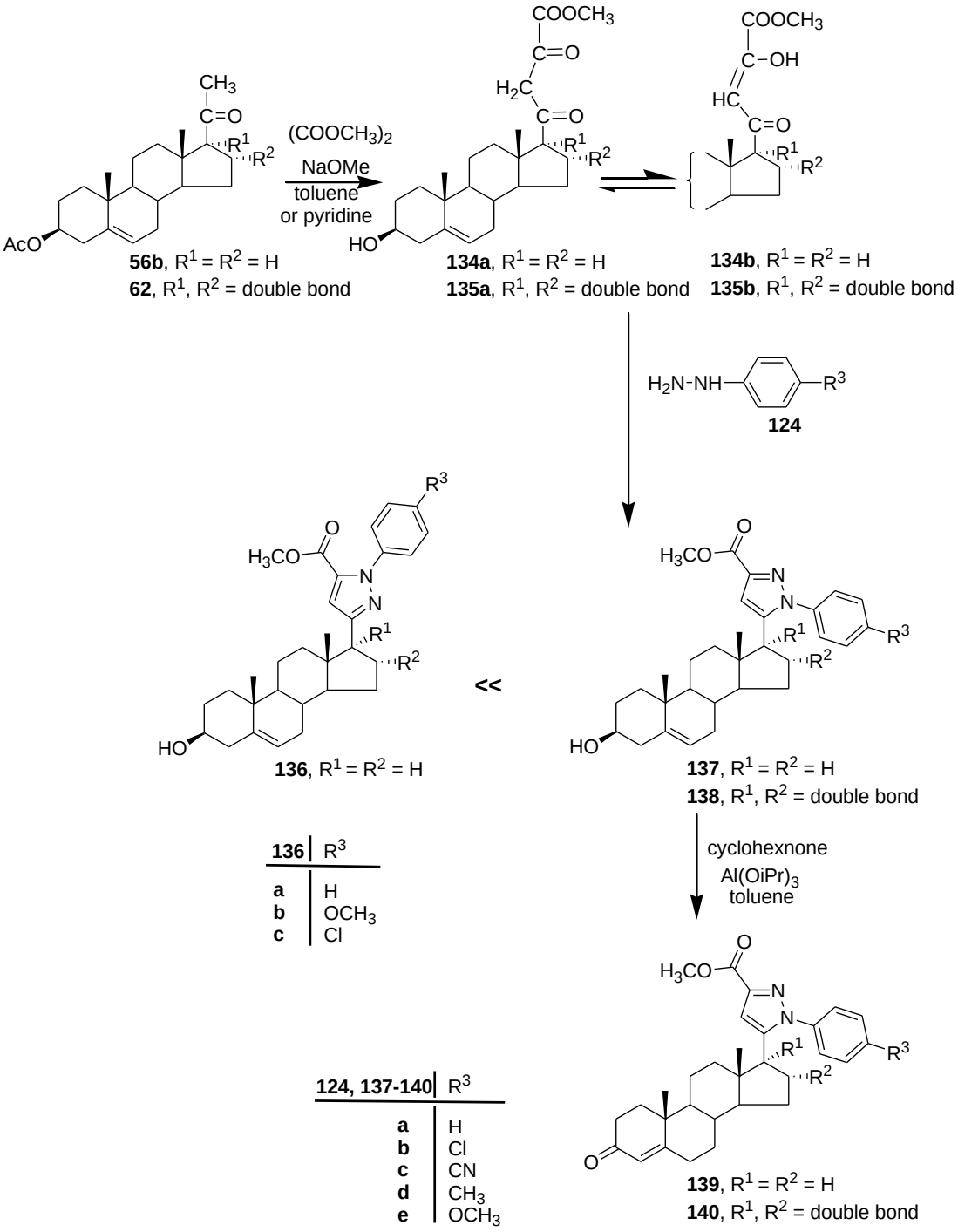


Scheme 3

3.8 The structures of the compounds prepared (**100a**–**e**, **101a**–**e**, **125a**–**e**, **126a**–**e**, **127a**–**e**, **128a**–**e**, **129a**–**e**, **130a**–**e**, **131a**–**e**, **132a**–**e**) were investigated with ^{1}H and ^{13}C NMR spectroscopic methods. The spectra verified the formation of the heterocycle in every case. Characteristic differences have been observed between the chemical shifts of the heteroaromatic protons in the two isomeric series, that are specific for the 1,3- and 1,5-disubstituted pyrazoles, this phenomenon confirmed the structure determination. Furthermore we established, that the 16-H signal of the regioisomeric androsta-5,16-dienes (**101a**–**e**, **126a**–**e**) also showed significant differences in the two series.

3.9 We extended our experiments for the synthesis of methoxycarbonyl-pyrazolyl steroids. The synthesis of the corresponding synton equivalents was achieved by the *Claisen* condensation of pregnenolone acetate (**56b**) or pregnadienolone acetate (**62**) with dimethyl oxalate. It was observed that the conversation lead to the 3β-hydroxy-21-methoxalylpregn-5-en-20-one (**134**) in good yield. For the preparation of 3β-hydroxy-21-methoxalylpregna-5,16-dien-20-one (**135**), the experimental conditions used in the synthesis of 3β-hydroxy-21-hydroxymethylidenepregna-5,16-dien-20-one (**90**), proved to be applicable (Scheme 4).

3.10 By investigating the ring closure reactions of the synton equivalents bearing methoxycarbonyl group (**89** and **90**), we concluded that the main products were the 5-pyrazolyl regioisomers (**137a**–**e**, **138a**–**e**). The 3-pyrazolyl isomers were formed in lesser amounts and were only isolable in the minority of the cases (**136a**–**c**).

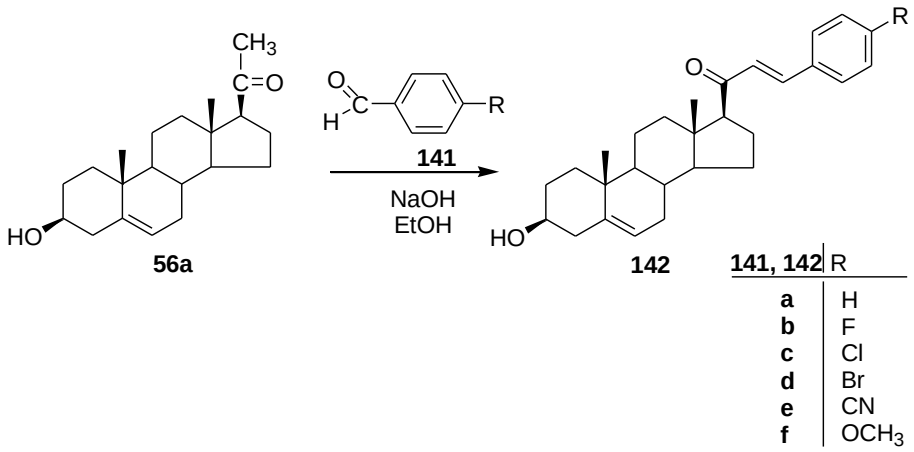


Scheme 4

3.11 The *Oppenauer* oxidation of the newly synthesized 3β-hydroxy derivatives **137a**–**e** and **138a**–**e** lead to the corresponding Δ^4-3-ketosteroides (**139a**–**e**, **140a**–**e**) in good yields.

3.12 The structure of the methoxycarbonylpyrazoles (**136a**–**c**, **137a**–**e**, **138a**–**e**, **139a**–**e**, **140a**–**e**) was proved by NMR spectroscopy, and we established that the ^{1}H NMR signal of the ester methyl group, and the C-4' signal show considerable differences in the two isomeric series.

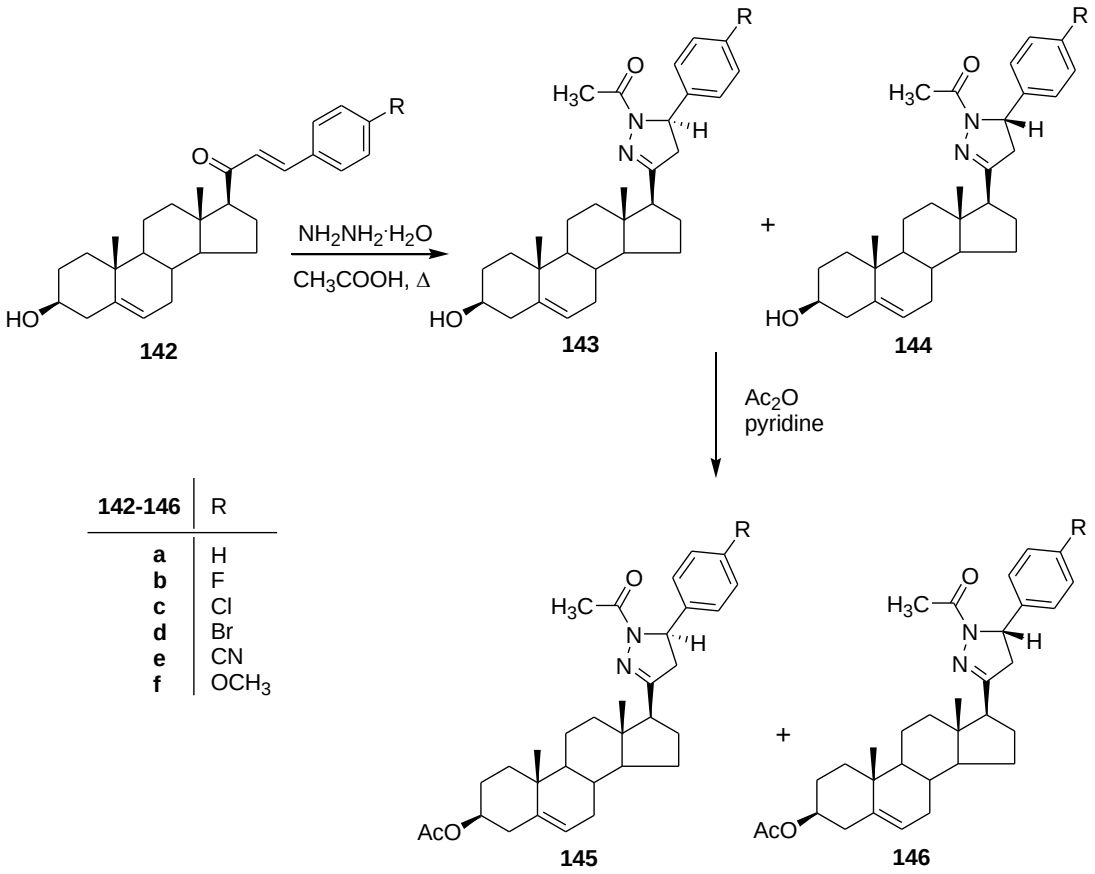
3.13 Further syntheses starting from pregnenolone (**56a**) lead to 21-benzylidene derivatives (**142a**–**f**), those were obtained by aldol condensation in alkaline solution. NMR spectroscopic investigation of the compounds proved the *E* geometry of the double bond in position 21 (Scheme 5).



Scheme 5

3.14 The ring closure reactions were performed with differently substituted benzylidenes (**142a**–**e**) and hydrazine hydrate. The reaction lead to an epimeric mixture of 5'*R* and 5'*S* pyrazolinylandrostenes (**143a**–**f**, **144a**–**f**), despite of the earlier literature observation. We determined the ratio of the isomers, and it was found to be 1:2 (*R*/*S*), independently from the substituent in the *p*-position of the starting material (Scheme 6).

3.15 The separation of the critical isomer pairs was achieved by acetylation of the crude reaction mixtures and chromatographic separation of the resulting 3β-acetates (**145a**–**f**, **146a**–**f**). The separated acetates were converted into their 3β-hydroxy (**143a**–**f**, **144a**–**f**) derivatives by the *Zemplén* method.



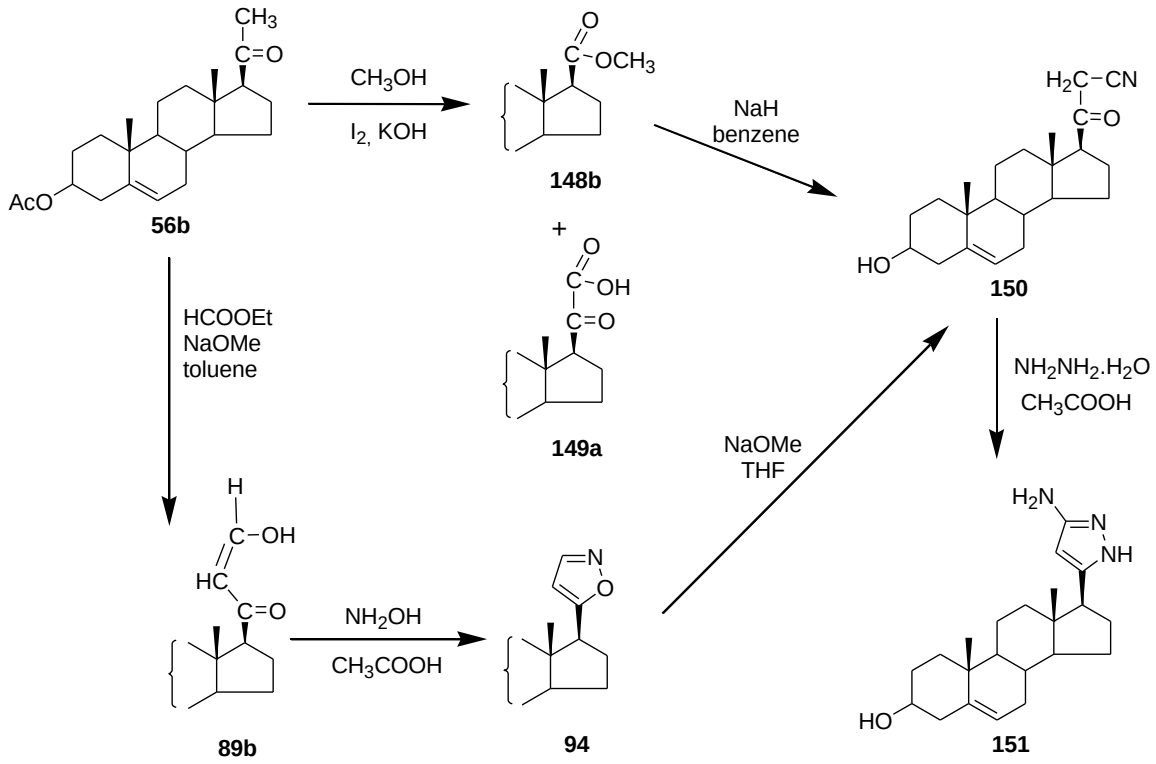
Scheme 6

3.16 We compared the NMR spectra of the separated isomers, and we found that the proton signals of the 4'-CH_2 groups show consequent differences. By 2D NMR spectroscopic investigations (COSY, HSQC, NOESY) of this observation, the structures of the isomers were successfully established. This was done by analyzing the NOE-distance relationships between the protons of the 4'-CH_2 and 5'-H, the phenyl group and protons belonging to the steroid skeleton.

3.17 We examined the possibilities for the synthesis of a new type of synton equivalent, the 3β-hydroxy-21-cyanopregn-5-en-20-one (**150**). Two synthetic methods starting from pregnenolone acetate (**56b**) have been compared. According to the first method, the starting material was converted into 3β-hydroxy-ethiochol-5-enic acid methyl ester (**148b**), then this was reacted with acetonitrile in the presence of NaH. During the second method, by reacting the previously prepared 3β-hydroxy-21-hydroxymethylidenepregn-5-en-20-one with hydroxylamine hydrochloride isoxazole **94** was obtained, which was opened with base (Scheme 7). Reaction of the resulting

compound (**150**) with hydrazine hydrate lead to the 17β-[3(5)-amino-5(3)-pyrazolyl]androst-5-en-3β-ol (**151**).

3.18 We examined the contradictions of the literature according to the preparation of the 3β-hydroxy-ethiochol-5-enic acid by haloform reaction. We found that the conversation in methanol lead to the methyl ester of the 3β-hydroxy-ethiochol-5-enic acid (**148b**), and to the unexpected 3β-hydroxypregn-5-en-20-one-21-carboxylic acid (**149a**). The same reaction in a mixture of dioxane and water also gave a considerable amount of 3β-hydroxypregn-5-en-20-one-21-carboxylic acid (**149a**) beside the ethiocholenic acid despite the observation in the literature.



Scheme 7

4. Papers forming the basis of the dissertation

1. Synthesis of regioisomeric 17β-*N*-phenylpyrazolyl steroid derivatives and their inhibitory effect on 17α-hydroxylase/$C_{17,20}$-lyase
   Zoltán Iványi, János Wölfling, Tamás Görbe, Mihály Szécsi, Tibor Wittmann, Gyula Schneider
   Steroids **2010**, *75*, 450–456.
   Impact factor (2010): **3.106**

2. Regioizomer androszt-5-én-, és androszta-5,16-dién vázas *N*-fenil-pirazolok szintézise (Synthesis of regioisomeric *N*-phenylpyrazolyl derivatives of androst-5-ene and androsta-5,16-diene)
   Zoltán Iványi
   Magyar Kémikusok Lapja **2010**, *LXV*, 391–393.
   Impact factor: –

3. Synthesis of D-ring-substituted (5'*R*)- and (5'*S*)-17β-pyrazolinylandrostene epimers and comparison of their potential anticancer activities.
   Zoltán Iványi, Nikoletta Szabó, Judit Huber, János Wölfling, István Zupkó, Mihály Szécsi, Tibor Wittmann, Gyula Schneider
   Steroids **2012**, *77*, 566–574.
   Impact factor (2011): **2.829**

4. Novel series of 17β-pyrazolylandrosta-5,16-diene derivatives and their inhibitory effect on 17α-hydroxylase/$C_{17,20}$-lyase
   Zoltán Iványi, Nikoletta Szabó, János Wölfling, Mihály Szécsi, János Julesz, Gyula Schneider
   Steroids, **2012**, *77*, 1152–1159.
   Impact factor (2011): **2.829**

Total impact factor for the publications that have already appeared: 8.764

5. Scientific lectures and posters forming the basis of the dissertation

Lectures:

1. Egy szokatlan jodoform reakció a pregnánvázas vegyületek sorában
   (An unusual iodoform reaction in the pregnane series)
   Zoltán Iványi
   A Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány 8. tudományos előadóülése
   Szeged, 16 April 2008.

2. Öttagú exo-heterociklusos szteroidok szintézise (nívódíjas előadás)
   (Synthesis of five-membered *exo*-heterocyclic steroids)
   Zoltán Iványi
   XXXI. Kémiai Előadói Napok
   Szeged, 27–29 October 2008.
   Program és előadás-összefoglalók 45–46. o.

3. An unusual iodoform reaction in the pregnane series
   Zoltán Iványi
   The 13th International Symposium for Students in Chemistry
   Timişoara, 21–22 November 2008.

4. 17β-*N*-fenil-pirazolil szteroidok előállítása
   (Synthesis of 17β-*N*-phenyl-pyrazolyl steroids)
   Zoltán Iványi
   A MTA Szteroidkémiai Munkabizottságának éves ülése
   Szeged, 27 November 2008.

5. Regioizomer androszt-5-én- és androszt-5,16-dién vázas *N*-fenil-pirazolok szintézise (Synthesis of regioisomeric *N*-phenylpyrazolyl derivatives of androst-5-ene and androsta-5,16-diene) (1^{th} prize)

   Zoltán Iványi

   A Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány 10. tudományos előadóülése

   Szeged, 5 May 2010.

Posters:

1. Regioselective syntheses of some 17β-*N*-phenyl-carbmethoxy-pyrazolyl steroids
   Zoltán Iványi, Gyula Schneider, János Wölfling
   COBC 2009 - German-French-Hungarian Congress in Organic and Biomolecular Chemistry
   Budapest, 20–23 June 2009.
   Book of Abstracts P-7, p. 43.

2. Syntheses of some 17β-*N*-phenylpyrazolyl steroids as inhibitors of human 17α-hydroxylase/$C_{17,20}$-lyase
   Zoltán Iványi, Gyula Schneider, János Wölfling, Mihály Szécsi
   JMMC 2009 – Joint Meeting on Medicinal Chemistry
   Budapest, 24–27 June 2009.
   Book of Abstracts P-27, p. 95.

3. Androszt-5-én és androszta-5,16-dién vázas pirazolok szintézise
   (Synthesis of pyrazolyl derivatives of androst-5-ene and androsta-5,16-diene)
   Zoltán Iványi, Gyula Schneider, János Wölfling
   Vegyészkonferencia és 53. Magyar Spektrokémiai Vándorgyűlés
   Hajdúszoboszló, 30 June – 2 July 2010.
   Program és előadás-összefoglalók, 114. o.

4. Epimer 17β-pirazolinil szteroidok szintézise
   (Synthesis of epimeric 17β-pyrazolinyl steroids)
   Nikoletta Szabó, **Zoltán Iványi**, János Wölfling, Gyula Schneider
   MKE 1. Nemzeti Konferencia
   Sopron, 22–25 May 2011.
   Program és előadás-összefoglalók, 206. o.

6. Papers not forming the base of the dissertation

1. Synthesis of steroidal dihydrooxazines and 2-oxazolidones, as novel potential inhibitors of 17α-hydroxylase-$C_{17,20}$-lyase
   Dóra Ondré, Gyula Schneider, **Zoltán Iványi**, István Tóth, Mihály Szécsi, János Julesz, János Wölfling
   1st Hungarian-Singaporean Workshop on Drug Discovery and Biomaterials
   Budapest, 10–11 March 2008.
   Proceedings, 98-100.
   Impact factor: –

2. Neighboring group participation, Part 17. Stereoselective synthesis of some steroidal 2-oxazolidones, as novel potential inhibitors of 17α-hydroxylase-$C_{17,20}$-lyase
   Dóra Ondré, János Wölfling, **Zoltán Iványi**, Gyula Schneider, István Tóth, Mihály Szécsi, János Julesz
   Steroids, **2008**, *73*, 1375–1384.
   Impact factor (2008): **2.588**

3. Synthesis and investigation of antiproliferative effect of estrone-16-oxime ethers
   Ágnes Berényi, Renáta Minorics, **Zoltán Iványi**, Imre Ocsovszki, Eszter Ducza, Hubert Thole, Josef Messinger, János Wölfling, Gergő Mótyán, Gyula Schneider, István Zupkó
   Steroids, (submitted for publication).

Total impact factor for the accepted publications not forming the base of the dissertation: 2.588

7. Posters not forming the base of the dissertation

1. Sztérikusan gátolt 17-azidoandroszt-5-én-3β-ol epimerek dipoláris cikloaddíciója
   (Dipolar cycloaddition of sterically hindered 17-azidoandrost-5-ene epimers)
   Tamás Görbe, **Zoltán Iványi**, Gyula Schneider, János Wölfling
   Vegyészkonferencia és 53. Magyar Spektrokémiai Vándorgyűlés
   Hajdúszoboszló, 30 June – 2 July 2010.
   Program és előadás-összefoglalók 113. o.

2. 17-Etinil-szteroidok 1,3-dipoláris cikloaddíciói
   (1,3-Dipolar cycloadditions of 17-ethynyl steroids)
   Zoltán Iványi, Éva Zsigó, Gyula Schneider, János Wölfling
   Vegyészkonferencia és 53. Magyar Spektrokémiai Vándorgyűlés
   Hajdúszoboszló, 30 June – 2 July 2010.
   Program és előadás-összefoglalók 146. o.

3. Egy szokatlan lefutású 1,3-dipoláris cikloaddíció a szteroidok sorában
   (An unusual 1,3-dipolar cycloaddition in the steroid series)
   Tamás Görbe, **Zoltán Iványi**, János Wölfling, Gyula Schneider
   MKE 1. Nemzeti Konferencia
   Sopron, 22–25 May 2011.
   Program és előadás összefoglalók 195. o.

4. Új 17β-*N*-fenil-pirazolil szteroid származékok gátló hatása az 5α-reduktáz 1-es tipusú izozim aktivitására
   (Inhibitory effect of novel 17β-*N*-phenyl-pyrazolyl steroid derivatives on the 5α-reductase type 1 isozime activity)
   Gábor Mahmoud, **Zoltán Iványi**, Tamás Görbe, Mihály Szécsi, János Wölfling, Gyula Schneider, Tibor Wittmann
   MKE 1. Nemzeti Konferencia
   Sopron, 22–25 May 2011.
   Program és előadás-összefoglalók 209. o.